28 VALPROIC ACID CONFIRMATION BY GCMS

28.1 Summary

28.1.1 Biological samples are slightly acidified with monosodium phosphate buffer (pH 5.5) and extracted with chloroform. An aliquot is injected into a GCMS for confirmation.

28.2 Specimen Requirements

28.2.1 1 mL biological fluid or comparable amount of tissue dilutions/homogenates

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28.3 Reagents and Standards

28.3.1 Phensuximide

28.3.2 Valproic acid

28.3.3 Monosodium phosphate (NaH_2PO_4)

28.3.4 Chloroform

28.3.5 Methanol

28.4 Solutions, Internal Standard, Calibrators and Controls

28.4.1 1 M monosodium phosphate (pH 5.5): Weigh 13 g monosodium phosphate (NaH_2PO_4) and transfer to a 100 mL volumetric flask. QS to volume with dH_2O.

28.4.2 20 mg/mL valproic acid stock solution. Weigh 200 mg valproic acid and transfer to 10 mL volumetric flask. QS to volume with methanol.

28.4.3 0.2 mg/mL phensuximide internal standard solution. Weigh 10 mg phensuximide and transfer to 50 mL volumetric flask. QS to volume with methanol.

28.4.4 Controls

28.4.4.1 Negative control. Blood bank blood (or comparable) determined not to contain valproic acid or phensuximide.

28.4.4.2 Positive control. In house control containing valproic acid spiked at concentration similar to case specimens.

28.5 Apparatus

28.5.1 Agilent GC/MSD, Chemstation software

28.5.2 Test tubes, 16 x 114 mm (10 mL) glass centrifuge, conical bottom

28.5.3 Centrifuge capable of 2000 – 3000 rpm

28.5.4 Vortex mixer

28.5.5 GC autosampler vials and inserts

28.5.6 GC/MSD parameters. Conditions may be changed to permit improved performance.

28.5.6.1 Acquisition Mode: Scan (50 – 550 amu)

28.5.6.2 Column: HP 5MS 25 m x 0.25 mm x 0.25 µm

28.5.6.3 Detector Temperature: 280° C

28.5.6.4 Oven Program

- Equilibration time: 0.50 minutes
- Initial temp: 110° C
- Initial time: 1 minutes
- Ramp: 10° C/min
- Final Temp: 280° C
- Final Time: 12 minutes
- Run Time: 28 minutes

28.5.6.4.1 Inlet

- Mode: Splitless
- Temperature: 260° C
- Injection volume: 1.0 µL
- Purge Time: ON at 1.0 minute

28.6 Procedure

28.6.1 Label clean 16 x 114 mm screw cap tubes accordingly, negative and positive control and case sample IDs.

28.6.2 Prepare negative and positive controls.

28.6.3 Pipet 1 mL of each case sample into appropriately labeled tubes.

28.6.4 Add 150 µL of 0.2 mg/mL phensuximide internal standard to each tube.

28.6.5 Add 1 mL sodium monophosphate buffer (pH 5.5) to each tube.

28.6.6 Add 1 mL chloroform to each tube.

28.6.7 Vortex briefly.

28.6.8 Centrifuge at approximately 2500 rpm for 15 minutes. Break protein plug and transfer bottom organic layer to appropriately labeled GC vials.

28.6.9 Inject 1 µl of each sample onto GCMS.

28.7 Calculation

28.7.1 Quantitative valproic acid results are determined by FPIA.

28.8 Quality Control and Reporting

28.8.1 See Toxicology Quality Guidelines

28.9 References

28.9.1 I. Sunshine. Methodology for Analytical Toxicology. CRC Press, 1982.